United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program

SOP No.: MDP-LABOP-02		Page 1 of 1
Title: Sample Receipt and Elution Procedure		
Revision: 03	Replaces: 08/15/03	Effective: 10/01/03

1. Purpose:

To provide standard procedures for receipt and washing of fruit and vegetable samples for the USDA/AMS Microbiological Data Program (MDP).

2. Scope:

This standard operating procedure (SOP) shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program.

3. Outline of Procedure:

5.1 Equipment and Materials

5.2 Media and Reagents

5.3 Receipt of Samples

5.4 Elution Method

4. References:

4.1 MDP LABOP-02, Sample Receipt and Elution Procedure Revision 02

4.2 SAMP-PROC-2, MDP Sampling Procedures on Site.

4.3 SAMP-PROC-3, Packing and Shipment of MDP Samples.

4.4 USDA/FSIS: SOP No. MLG Appendix 1.02; Rev.02, effective 7/3/03

4.5 Baylis CL, MacPhee S, and Betts RP. 2000. Comparison of two commercial preparations of buffered peptone water for the recovery and growth of Salmonella bacteria from foods. Journal of Applied Microbiology. 89: 501-510.

4.6 Andrews, W.H., Sherrod, P.S., Hammack, T.T., and Amaguana, R.R., 1998. Food and Drug Administration Bacteriological Analytical Manual (BAM), 8th ed. (revision A). George J. Jackson (Ed). AOAC International, Gaithersburg, MD 20877 pp. App 3.64

5. Specific Procedures:

5.1 Equipment and Materials

5.1.1 Balance, minimum 1000 g with minimum of 0.1 g sensitivity

5.1.2 Rotary or orbital shaker, set at 2.54 cm (1 inch) stroke

5.1.3 In-house adaptation for orbital shaker bed to shake cantaloupe samples

5.1.4 Plastic bags, sterile, suitable size to hold sample and eluent. e.g., sterile 3500 stomacher bag or sterile 12" x 12" Uline zipper-closure bags.

5.1.5 Forceps, tongs, slotted spoons, sterile

5.1.6 Thermometer, Raytek Portable IR Sensor, P/N Rayst20CRUS

5.1.7 Gloves, sterile

5.2 Media and Reagents

5.2.1 Buffered Peptone Water (BPW)

5.2.2 Sodium hydroxide (NaOH) solution for pH adjustment

5.2.3 Hydrochloric acid (HCl) for pH adjustment

5.2.4 Buffered Peptone Water plus 0.1% Tween 80

Prepare buffered peptone water plus 0.1% Tween 80 (v/v) for use as eluent

Peptone	10.0 g
Sodium chloride	5.0 g
Sodium phosphate, dibasic	3.5 g
Potassium phosphate, monobasic	1.5 g
Tween 80	1.0 mL
Distilled water	1.0 L

Dissolve dry ingredients in distilled water, add 1.0 mL of Tween 80 and dissolve again. Adjust to a final pH of 7.2 ± 0.2 at room temperature (approximately between 22-25°C) using NaOH or HCl. Commercially prepared dehydrated media may also be used. The media must be prepared according to manufacturer's instructions with a final concentration of 0.1% Tween 80 in BPW. Dispense into appropriate containers and autoclave at 120 ±1°C for 15 minutes. Commercially prepared liquid media may also be used.

5.3 Receipt of Samples

5.3.1 The laboratory will receive 3 samples (or multiples thereof) of the same produce in each shipping container. Upon arrival at the laboratory, take the temperature of all 3 samples and record the date and time of sample receipt.

5.3.2 Determine the temperature of the produce by pointing and activating the IR thermometer at the surface of each sample. **Do not** take the temperature through the plastic bag. Carefully open a sample bag to obtain direct access to the surface of the sample. The bags should be sealed in such a way that they can be opened and re-sealed easily. If this is not the case, contact your sampling manager to arrange for appropriate modifications in bag closure procedures. Do not touch the produce with bare or gloved hands unless or until they are removed from the bag for transfer to other sterile bags for weighing and addition of BPW plus 0.1% Tween 80.

5.3.2.1 **Lettuce samples**: Take the temperature midway from top to bottom on a leaf.

5.3.2.2 **Celery Samples**: Take the temperature midway from top to bottom on the bunch.

5.3.2.3 **Other Commodities**: Take the temperature at any point on the produce

5.3.3 Test all laboratory samples regardless of temperature unless extensive spoilage or freezing has taken place.

5.3.4 Refrigerate the samples until analysis begins. Perform the analysis as soon as realistically possible but no more than 24 hours after receipt in the laboratory.

5.4 Elution Method

5.4.1 Perform all manipulations using sterile technique.

5.4.2 Tare a sterile bag and add sample to bag. Do not composite samples. Each sample is washed and tested individually.

5.4.2.1 **Tomatoes**: Test only whole tomatoes. Do not remove labels, stems or leaves. Each tomato sample should weigh at least 100 g. Do not test tomatoes with cracks or breaks in their surface.

5.4.2.2 **Lettuce**: Test approximately 200 g of outer lettuce leaves. Remove and discard any outer leaves that exhibit freezing damage, obvious wilt, decay or that have obvious clumps of dirt clinging to them. Aseptically pull whole leaves of lettuce from the base of the loose-leaf lettuce. Test only whole leaves of lettuce, but do not reject lettuce leaves that are torn or have breaks in their surface.

5.4.2.3 **Celery:** Select enough stalks to obtain approximately 200 g per sample. Aseptically pull whole stalks from the base of the bunch of celery (include leaves, if present). Test only whole stalks of celery. Do NOT test stalks of samples that show spoilage or are severely damaged.

5.4.2.4 **Cantaloupe**: Test each of the 3 cantaloupe samples individually. Test only whole cantaloupes. Do not reject cantaloupes with fresh, minor surface damage. Do not test cantaloupes that show spoilage or are severely damaged.

5.4.4 Add sterile buffered peptone water containing 0.1% Tween 80 as eluent to bag with sample.

5.5.4.1 **Lettuce**: Add a weight of eluent equal to the weight of lettuce to the bag (± 5 g)

5.5.4.2 **Tomatoes**: Add a weight of eluent equal to the weight of tomatoes to the bag (±5 g)

5.5.4.3 **Celery**: Add a weight of eluent equal to the weight of celery to the bag (± 5 g)

5.5.4.4 **Cantaloupes**: Add a weight of eluent equal to one-quarter the weight of the cantaloupe (± 5 g).

5.4.5 Seal bags so that no fluid can leak when the bag is placed on the shaker

5.4.6 Place sealed bag on rotating shaker and shake the samples at 160 rpm, stroke size 2.54 cm (1 inch) for 7 minutes. Turn sample bag over after half the time has passed and continue shaking.

5.4.6.1 **Cantaloupes**: Place bagged cantaloupes in round containers on shaker adaptation. Shake as in 5.4.6.

5.4.7 Use the eluate as the sample. Proceed with subsequent cultural analyses according to the SOPs for those analytes.

5.4.8 Discard excess eluate and the original sample after testing begins.

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Revision 03	September 2003	Monitoring Programs Office

- Updated references
- Changed wash buffer from 1.0% Tween 80 in Butterfield's phosphate buffer to buffered peptone water with 0.1% Tween 80

Fabrication of the California Cantaloupe Shaker Adapter For mechanical elution of surface bacteria from cantaloupes.

Overview: Several 5-quart plastic pails are glued to a plywood sheet which is screwed to the top metal bed plate of the orbital shaker. Cantaloupes in double plastic bags with Butterfield's-Tween are shaken according to the draft Cantaloupe Sample-Wash MDP SOP. (See CN Photo #1) *First suggested on 2/6/02 by Dwight Harder, Director of the Arizona Agriculture Laboratory.*

1. Purchase or collect a few empty, clean 5-quart plastic pails, with relatively vertical sides. These are commonly used for ice cream (vanilla is good with cantaloupe). They can also be purchased from a hardware store, Target, Wal-Mart, etc. Determine the arrangement of several pails to be lined up contiguously on a sheet of 3/8", ½" or 5/8" thick plywood approximately the dimensions or larger of the bed of your orbital shaker. Circumscribe the circumference of the pails in the position where they will be later glued (i.e, draw a circle around each pail). See CN Photo #2. Do not use "chip board", "particle board" or "wafer board". It is good to remove the metal or plastic bails (handles) from the pails as they will chatter during shaking.

2. Cut plywood to accommodate the number of pails you will glue to the plywood. California has 12 pails (4 rows of 3 ea) on a plywood board measuring 23" by 32" for our shaker which has an 18" x 30" bed.

3. The plywood must be screwed to the metal bed of the shaker, and able to be removed on the days commodities other than CNs are washed. If possible, remove the metal top bed plate from the shaker. California's is attached to the shaker with 4 large slotted screws. Use a large enough screwdriver so as not to strip the screw slots. Position the removed shaker plate upside down on the upside-down plywood and mark at least 4 predrilled and threaded holes in the shaker plate to align and drill holes through the plywood. Be sure screw holes will not be obstructed by plastic pails later, to ensure convenient removal of the CN Shaker adapter from

the shaker bed plate. Reassemble bed plate back onto the shaker. This is a good time to perform inspection preventive maintenance and belt-tightening of the shaker, if needed.

4. Obtain machine screws of proper length, diameter, and thread pitch to screw the plywood sheet to the metal shaker bed plate. Use washers, and be sure the screws are tight and do not protrude through the shaker bed plate to obstruct the shaking function. (See CN photo #3)

5. Glue plastic pails to the plywood in the locations previously marked using construction adhesive, subfloor adhesive, or "Liquid Nails". This is sold by hardware stores and building-supply outlets (Home Depot, Lowes) in 10.5 Fl.Oz. "caulking" tubes (< $3.00). You will also need a "caulking" gun (<< $5.00). Tight adhesion is guaranteed by using plenty of goo, and scooting the pails side-to side prior to their final location and setting of the adhesive. Tight adhesion is facilitated by weighting each pail down with 2-4 quarts of water (in plastic bags) during the 1-2 day curing of the adhesive. Work in a well

ventilated area and do not use so much goo that large quantities ooze from the sides of the bases of the pails. Although his is usually not a problem, it is wise to initially try your adhesive with one of your specific pails to determine if the volatile organic solvent in the adhesive softens the plastic of the pail.

6. Test your Cantaloupe adapter and shaker in advance of receiving melons by placing a volume of water in plastic bags in each pail equivalent to a melon plus an equal volume by weight of water. Large cantaloupes weigh about 1.2 Kg, so 2.4 liters of water would be a good volume for the test. Such weights, more than other commodities, will cause the shaker to shimmy. Be sure it is secure. California's is on wheels, which must be stabilized.

NOTE: The California MDP Lab., in collaboration with the Food Safety & Health unit of USDA's ARS-WRRC in Albany, CA is microbiologically validating the elution efficiency of this method for the MDP. Results are very promising, and will be forthcoming soon to MDP, in advance of publication. This is the most promising and feasible method for MDP of the 4 we have evaluated.